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**GANGLIOSIDE PATTERN AND NEURAMINIC ACID CONTENT OF HUMAN GASTRIC AND COLONIC CARCINOMA**ANTTI KERÄNEN <sup>a,\*</sup>, MATTI LEMPINEN <sup>b</sup> and KARI PURO <sup>a</sup><sup>a</sup> *Department of Medical Chemistry, University of Helsinki, Siltavuorenpenger 10 A, SF-00170 Helsinki 17,* <sup>b</sup> *II Department of Surgery, Central Hospital, University of Helsinki, Helsinki (Finland)*

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**Summary**

Twenty-five human gastric and 11 human colonic adenocarcinomas were analysed for their ganglioside pattern and for their content of lipid-bound and protein-bound neuraminic acid. In most carcinomas the content of both lipid-bound and protein-bound neuraminic acid was increased by an average of four- and two-fold, respectively. The ganglioside pattern of all the carcinomas resembled that of normal tissue. In six gastric carcinomas the content of lipid-bound neuraminic acid and the ratio of lipid-bound neuraminic acid to protein-bound neuraminic acid (L/P ratio) were lower than those of normal gastric mucosa. These carcinomas were significantly larger than the rest of the tumours.

**Introduction**

Ever since Hakomori and Murakami [1] demonstrated glycolipid changes in fibroblasts of baby hamster kidney and the derived spontaneous or polyoma virus transformants, variously transformed cells have been the subject of vigorous research. The studies have recently been reviewed extensively by Hakomori [2] and Brady and Fishman [3]. In chemically or virally transformed cells there seems to be a block in the synthesis of gangliosides; this block causes a simplification of the ganglioside pattern [2,3].

The interesting results obtained in the studies with cultured cells prompted us to consider whether changes could also be found in human malignant tumours. To date, only a few studies dealing with this problem have been published. In the most recent report, Karlsson et al. found only haematosides in

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human kidney [4]. Unfortunately the normal ganglioside composition of human kidney is not known, and therefore possible changes in the ganglioside pattern could not be detected.

Changes in the content of neuraminic acid have also been described for both transformed cells and solid tumours. In virally transformed cells a decreased content of neuraminic acid has been found [5-8], whereas human kidney carcinoma [4] and human leukaemic leucocytes [9] have shown an increased content of sialic acid.

In an earlier study we were able to detect nine different gangliosides in the normal human gastrointestinal mucosa [10]. Both haematosides and sialotetraglycosylceramides were present. We also found differences in the content of both the lipid-bound and protein-bound neuraminic acid of gastric, small intestinal and colonic mucosa [10].

To determine whether the ganglioside pattern and neuraminic acid content of human malignant tissue differs from that of normal tissue, we analysed the ganglioside pattern of 25 human gastric and 11 human colonic adenocarcinomas, determined their neuraminic acid content and compared the results to those obtained from normal gastrointestinal mucosa [10].

## Material and methods

### *Tumour material*

The tumours were obtained from 25 patients operated on for gastric carcinoma and 11 patients operated on for colonic carcinoma. Small pieces of the tumours were examined for the histological diagnosis. All the tumours were of the adenocarcinoma type. Immediately after the operations the tumours were macroscopically separated from the normal tissue and washed with +4°C saline. All macroscopically visible necrotic parts of the tumour were also removed. Gastric tumours weighed from 16.5 to 330.0 g (mean  $\pm$  S.D. 106.6  $\pm$  76.3 g), and colonic tumours from 10.4 g to 191.7 g (mean  $\pm$  S.D., 56.0  $\pm$  51.0 g).

### *Reference material*

The reference tissue consisted of human gastric and colonic mucosa obtained from cadavers. The ganglioside composition of these tissues has been presented in detail elsewhere [10]. To ensure that the autopsy material is comparable to material obtained from living persons, we also analysed stomachs resected because of gastric ulcer. The ganglioside composition and neuraminic acid content of the resected sections were identical with those of the autopsy material.

### *Extraction, purification and characterisation of the gangliosides*

The methods used have been presented in detail elsewhere [10]. Lipids were extracted from the tissue with a chloroform/methanol mixture. The extracts were partitioned with water and the upper phases dialysed against running tap water. The gangliosides were purified with cellulose [11] and silicic acid [12] column chromatography. Thin-layer [13] and gas-liquid [14,15] chromatography were used for the characterisation of the gangliosides.

### Quantitative analytical methods

Neuraminic acid was determined according to the method of Svennerholm [16,17] as modified by Miettinen and Takki-Luukkainen [18]. In the determination of lipid-bound neuraminic acid, chloroform-methanol extracts were used. The samples for the determination of total tissue neuraminic acid were obtained by the homogenisation of tissue in water. The values for protein-bound neuraminic acid were obtained by subtracting the values for lipid-bound neuraminic acid from those for total neuraminic acid.

Dry weight values were obtained by drying tissue samples to constant weight at 100°C.

The relative amounts of different gangliosides in a carcinoma were determined with the thin-layer chromatography method of Suzuki [19] as modified by Keränen [10].

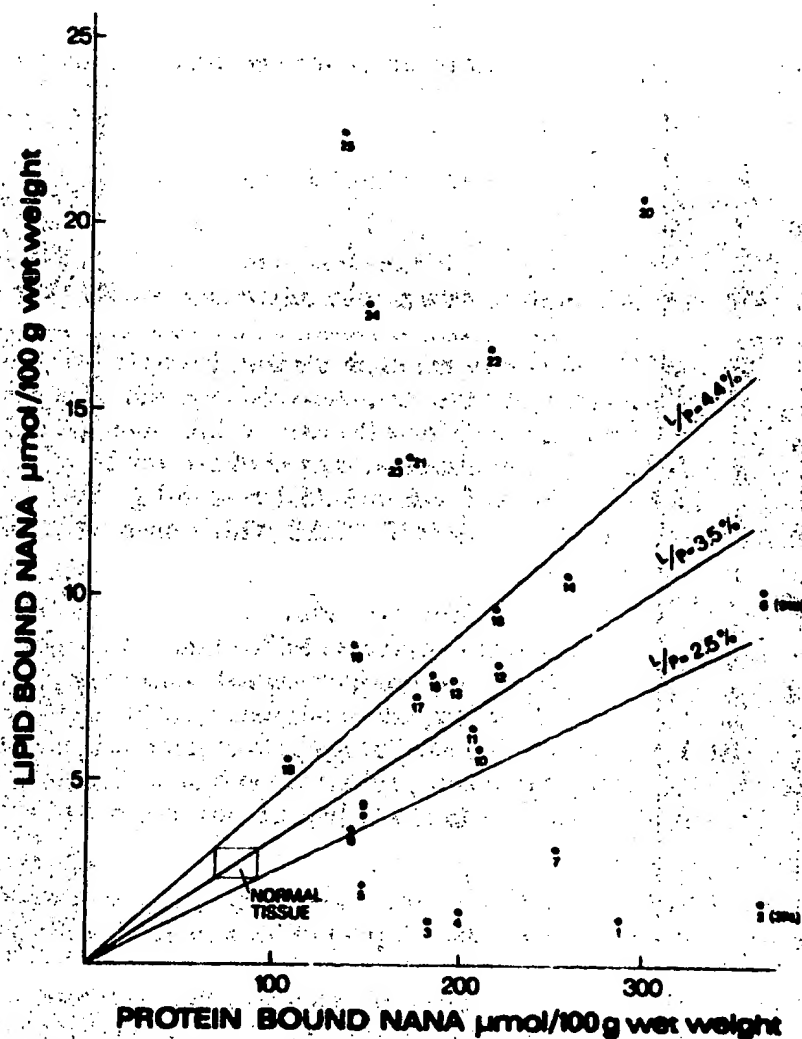


Fig. 1. Content of lipid- and protein-bound neuraminic acid in 25 human gastric cancers. Numbered dots refer to single cancers (see Table I). L/P, percentage ratio of lipid-bound neuraminic acid content to protein-bound neuraminic acid content.

## Results

### Gastric cancer

Twenty-five human gastric carcinomas were analysed. The results are presented in Fig. 1 and Table I.

**Content of lipid-bound neuraminic acid.** The amount of gangliosides in the gastric carcinomas varied greatly. Most of the carcinomas had a higher content, up to 22.6  $\mu\text{mol}$  of *N*-acetylneuraminic acid per 100 g wet weight, than normal human gastric mucosa. On average, the ganglioside content in the carcinomas was four-fold that of normal tissue.

In five carcinomas the amount of gangliosides was approx. half of that of normal tissue.

**Thin-layer chromatographic analysis.** The ganglioside patterns of the carcino-

TABLE I

### CONTENT OF LIPID-BOUND AND PROTEIN-BOUND NEURAMINIC ACID IN 25 HUMAN GASTRIC CANCERS

The numbers of the cancers correspond with those in Fig. 1.

No.	Tumour weight (g)	Neuraminic acid, expressed as NANA				L/P (%)
		$\mu\text{mol}/100 \text{ g of wet weight}^{\dagger}$		$\mu\text{mol}/100 \text{ g of dry weight}$		
		Lipid-bound	Protein-bound	Lipid-bound	Protein-bound	
1	140.0	1.2	288	6.4	1493	0.4
2	107.0	1.7	374	7.6	1723	0.4
3	124.0	1.1	184	6.0	993	0.6
4	330.0	1.4	201	7.2	1059	1.7
5	100.0	2.1	148	10.4	751	1.4
6 *	284.4	10.2	918	80.9	7687	1.1
7	159.0	3.1	253	17.9	1437	1.2
8	93.0	3.6	142	18.4	735	2.5
9	104.0	4.0	149	21.0	777	2.5
10	152.0	5.8	211	30.6	1111	2.8
11	71.0	6.3	208	36.0	1192	3.0
12	25.5	6.1	221	34.2	931	3.7
13	145.0	7.6	197	43.2	1126	3.9
14	66.1	10.5	258	52.7	1296	4.1
15	24.5	7.8	185	29.4	697	4.2
16	63.8	9.6	220	43.7	1001	4.4
17	152.0	7.2	177	36.9	810	4.1
18	91.0	5.5	108	25.6	505	5.1
19	126.0	8.6	143	47.8	796	6.0
20	96.0	20.8	299	121.0	1769	7.0
21	36.0	13.7	171	61.7	773	8.0
22	72.0	16.7	208	53.8	668	8.1
23	42.0	13.6	166	58.3	711	8.2
24	34.0	17.9	150	93.8	782	12.0
25	16.5	22.6	136	103.5	623	16.6
Normal tissue **		2.7 $\pm$	81 $\pm$	16.8 $\pm$	494 $\pm$	3.5 $\pm$
		0.4	11	2.6	63	0.8

\* Tumour containing large amounts of mucus.

\*\* Values are expressed as mean  $\pm$  S.D. of 20 samples (see ref. 10)

mas were compared to normal patterns with thin-layer chromatography. All nine gangliosides of normal gastric mucosa [10] were detected in each tumour sample. A great similarity can clearly be seen between the ganglioside patterns of the normal and malignant tissue on thin-layer plates (Fig. 2). All the complex mono- and disialotetraglycosylceramides, both galactosamine and glucosamine containing, were present in an approximately normal amount. According to the quantitative analysis the relative amounts of different gangliosides were approximately normal.

**Content of protein-bound neuraminic acid.** The amount of protein-bound *N*-acetylneuraminic acid (NANA) was higher than normal in all the samples. On average, the content was 2.3-fold that of the normal tissue and ranged from 108  $\mu\text{mol}$  to 374  $\mu\text{mol}/100$  g wet weight, mean  $\pm$  S.D.  $200 \pm 61$   $\mu\text{mol}$  g wet weight. Carcinoma No. 6 was omitted because of its extremely high content of protein-bound NANA, 918  $\mu\text{mol}/100$  g wet weight, a finding which agreed with

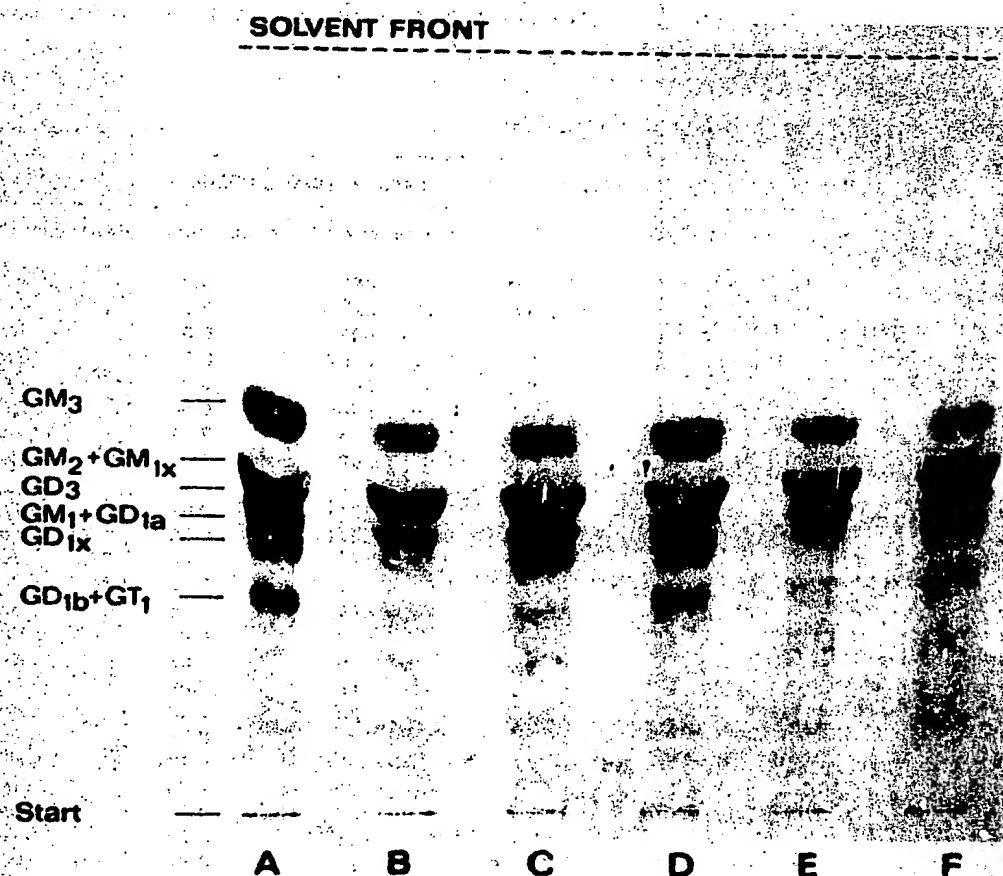


Fig. 2. Thin-layer chromatogram of gangliosides obtained from human gastric carcinomas. A, normal gastric mucosa. Cancers: B, No. 7; C, No. 10; D, No. 15; E, No. 19; F, No. 22 (see Table I). The shorthand nomenclature for gangliosides worked out by Svennerholm [35] was used.  $\text{GM}_{1x}$  is mononeuraminyltetraglycosylceramide containing glucosamine and  $\text{GD}_{1x}$  is dineuraminyltetraglycosylceramide containing glucosamine. The developing solvent was *n*-propanol/concentrated ammonia/water (6 : 1 : 2, v/v/v); the spray was resorcinol.

the high content of mucus seen in the histological examination. To find out if there was any correlation between the two parameters analysed above and tumour weight, both wet and dry, we performed a linear regression analysis (Table II). No correlation was found. When the carcinoma material was grouped according to the ratio of the lipid bound neuraminic acid content to the protein-bound neuraminic acid content (L/P ratio), three groups were obtained (Fig. 1). In the first group (group A), six carcinomas had an L/P ratio that was smaller than normal. The second group (group B) consisted of 11 carcinomas with an L/P ratio that was within normal limits,  $3.5 \pm 0.8\%$ . All other carcinomas belonged to the third group (group C) and had an L/P ratio that was larger than normal.

The mean tumour weights of the groups were tested against each other (Table II). An one-factor analysis of variance showed a significant difference between the groups ( $p < 0.025$ ). In Student-Newman-Keuls's test, group A differed significantly ( $p < 0.05$ ) from the groups B and C. The difference between groups B and C was not significant. On the basis of this statistical analysis, it can be concluded that tumours in group A are significantly bigger than the rest of the tumours.

### Colonic cancer

Eleven colonic carcinomas were analysed. The results are presented in Table III and Fig. 3.

TABLE II  
WEIGHTS OF GASTRIC CARCINOMAS IN DIFFERENT L/P GROUPS

Group	Tumours	L/P (%)	Weight (g) (mean $\pm$ S.D.)	$p^*$	Correlation coefficients **				
					$r_1$	$r_2$	$r_3$		
A	1-7 §	<2.5	160 $\pm$ 86		0.63	-0.13	-0.20		
B	8-17	2.5 < and $\leq$ 4.4	90 $\pm$ 48	(A-B)	<0.05				
				(A-C)	<0.05	-0.27	-0.35	-0.27	
C	18-25	>4.4	64 $\pm$ 38	(B-C)	N.S.	-0.77	-0.53	0.21	
	1-25 §					-0.55	-0.56	0.16	
Statistical analysis ***									
Analysis of variance, one factor					Student-Newman-Keuls's test				
Source	SS	df	MS	F	p	Source	$d\bar{x}$	$D'$	p
Within	68077	21	3242			A-C	96	82	<0.05
Between	32887	2	16443	5.07	<0.025	A-B	71	63	<0.05
						B-C	25	57	N.S.

\* Student-Newman-Keuls's test.

\*\* Correlation coefficient, linear regression analysis;  $r_1$ , tumour weight to L/P;  $r_2$ , tumour weight to lipid bound neuraminic acid;  $r_3$ , tumour weight to protein-bound neuraminic acid.

\*\*\* Abbreviations used: SS, square sum; df, degrees of freedom; MS, mean square; F, F-ratio;  $d\bar{x}$ , difference of means;  $D'$ , result of the Student-Newman-Keul's test.

§ Tumour No. 6 omitted.

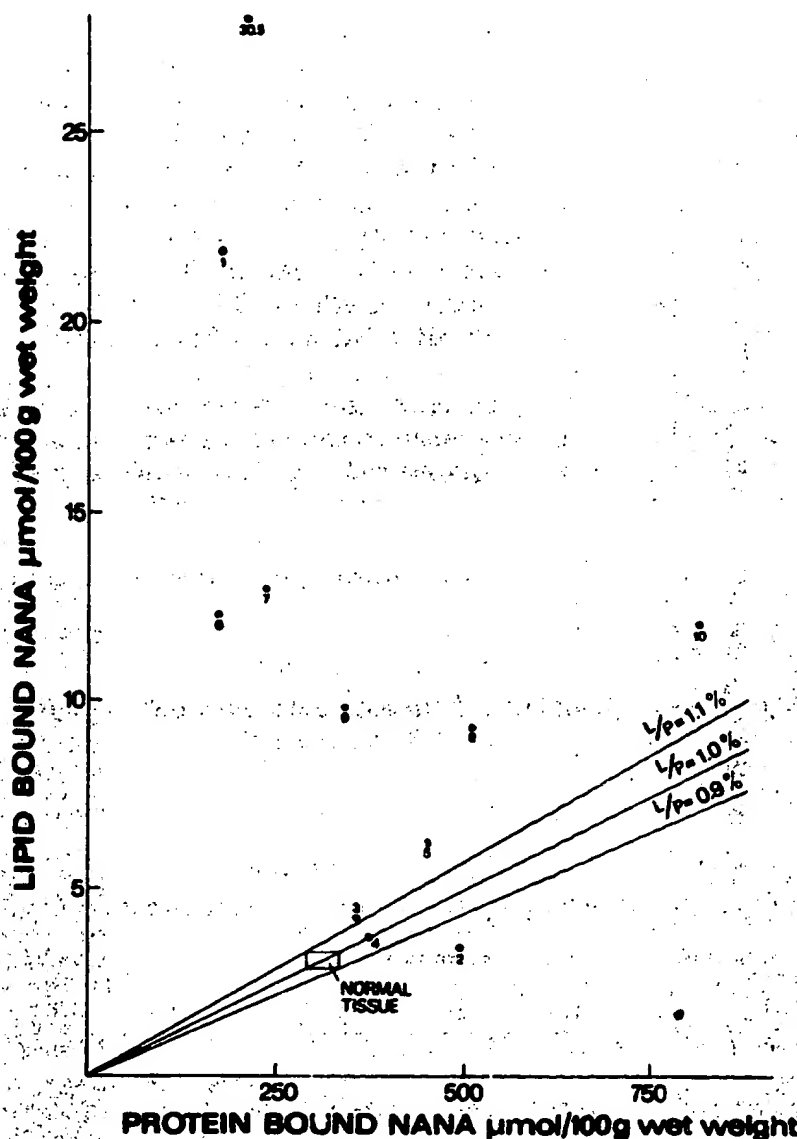


Fig. 3. Content of lipid- and protein-bound neuraminic acid in 11 human colonic cancers. For explanations see Fig. 1 and Table III.

**Content of lipid-bound and protein-bound NANA.** Lipid-bound NANA content was high in all colonic carcinomas and ranged from 3.4 to 30.5  $\mu\text{mol}/100$  g wet weight. The average content  $11.2 \pm 8.4$   $\mu\text{mol}/100$  g wet weight, was 3.7-fold that of normal tissue.

Protein-bound NANA content varied greatly, from 170 to 814  $\mu\text{mol}$  of *N*-acetylneuraminic acid per 100 g wet weight. The mean of protein-bound NANA content of total colonic material  $374 \pm 190$   $\mu\text{mol}/100$  g wet weight, did not differ statistically (*t*-test) from normal. Most carcinomas had an L/P ratio greater than normal. No grouping agreeing with this ratio was possible. There was no correlation (linear regression analysis) between the content of lipid-



TABLE III

## CONTENT OF LIPID- AND PROTEIN-BOUND NEURAMINIC ACID IN 11 HUMAN COLONIC CANCERS

The numbers of the cancers correspond with those in Fig. 3.

No.	Tumour weight (g)	Neuraminic acid, expressed as NANA				L/P (%)
		$\mu\text{mol}/100 \text{ g of wet weight}$		$\mu\text{mol}/100 \text{ g of dry weight}$		
		Lipid-bound	Protein-bound	Lipid-bound	Protein-bound	
1	71.8	21.9	174	95.3	759	12.5
2	49.0	3.4	492	14.0	2025	0.7
3	56.0	4.2	366	17.5	1489	1.2
4	43.0	8.7	373	15.5	1571	1.0
5	72.0	6.2	450	33.4	2418	1.4
6	25.0	12.3	170	35.0	485	7.2
7	76.4	13.0	231	35.6	636	5.6
8	10.4	9.3	508	43.5	2384	1.8
9	12.0	9.8	335	52.4	1789	2.9
10	191.7	12.1	814	50.8	3420	1.5
11	12.0	30.5	209	78.3	538	14.6
Normal tissue *		3.1 $\pm$	310 $\pm$	11.4 $\pm$	1148 $\pm$	1.0 $\pm$
		0.2	22	0.5	80	0.1

\* Values are expressed as mean  $\pm$  S.D. of 10 samples (see ref. 10).

bound and protein-bound neuraminic acid and tumour weight among different colonic carcinomas.

*Thin-layer chromatographic analysis.* The ganglioside pattern of all the colonic carcinomas seemed normal. All normal colonic gangliosides were present in relatively normal amounts.

### Discussion

In our study most of the gastric tumours and all of the colonic tumours contained approx. four times more lipid-bound neuraminic acid, i.e. gangliosides, than corresponding normal tissue. Karlsson et al. [4] reported a similar increase in the ganglioside content of human kidney carcinoma. The amount of gangliosides in leucocytes from patients with chronic myelogeneous leukaemia has also been found to be high [9]. No direct conclusions can be drawn from our results, but one explanation for the increased ganglioside content could be the reduced cell size of most adenocarcinomas, since a reduced cell size would mean a relatively increased amount of plasma membrane in a weight unit. If the density of lipid-bound neuraminic acid in the plasma membrane is normal or increased, as has been described for protein-bound neuraminic acid in several transformed cells [26], the amount of lipid-bound neuraminic acid in the tissue would increase.

In all the tumours we analysed the ganglioside pattern seemed normal. This finding does not agree with those reported for different virally or chemically

transformed cell lines [2,3]. On the other hand, Mora et al. [20] found a normal ganglioside pattern in cell spontaneously transformed in vitro to a tumorigenic state, a finding contradictory to the original observation of Hakomori and Murakami [1]. Thus, the question of whether the changes in the ganglioside pattern observed in cells transformed in vitro are an expression of the tumorigenicity of the cell or of the virus transformation remains to be elucidated. At this time the latter possibility has received the most support [2, 3].

Only a few studies have been made of human malignant tissues. In human glial tumour only mono- and dineuraminyl haematosides were found [21,22]. In human kidney carcinoma [4] only a mononeuraminyl haematoside was detected. Since the normal ganglioside composition of these tissues is not known, no change in the ganglioside pattern could be determined.

In our study we did not find any differences between the ganglioside composition of human gastric and colonic adenocarcinomas and corresponding normal mucosae. Only the total amount of gangliosides was increased in malignant tissues.

The amount of protein-bound neuraminic acid was higher than normal in all the gastric carcinomas we examined. In the colonic carcinomas it was high in most, but low in some. The amount of protein-bound NANA in normal colonic mucosa was four times higher than that found in normal gastric mucosa [10]. In our carcinoma material this kind of tissue specificity was lost. The means of the protein-bound neuraminic acid content of the gastric and colonic carcinomas did not differ statistically (*t*-test).

A decreased amount of total neuraminic acid has usually been found in virally transformed cells [5-8]. In human colonic adenocarcinoma the amount of membrane sialopeptides was decreased when compared to adjacent normal mucosa [23]. Thus, neither elevated sialyltransferase activity [24,25] nor increased sialic acid density in surface glycoprotein [26], two findings observed in virally transformed cells, can explain our finding.

In mucus-secreting cells, such as gastric and colonic mucosal cells, a block in the secretory mechanism leads to increased sialoprotein, and thus to an increased NANA content, if the half-life of unsecreted sialoproteins is not respectively shortened. We suppose that such an occurrence could cause the increased protein-bound NANA content in gastric and colonic tumours. Supporting evidence has been presented by Harms et al. [27,28]. They found an elevated NANA content in Morris hepatomas, in which the half-life of the NANA in intracellular sialoproteins was considerably longer than the half-life of the plasma-membrane-bound NANA. On the other hand, Schreiber et al. [29] have shown that some hepatomas lack the ability to secrete plasma proteins.

The participation in both glycolipid and glycoprotein neuraminic acid in cell-contact phenomena and growth control has already been demonstrated [30-34]. We chose the ratio of lipid-bound neuraminic acid content to protein-bound neuraminic acid content as the common denominator for the grouping of our carcinoma material. This ratio includes both the parameters found to change in malignant transformation, glycolipid and glycoprotein content. The six gastric carcinomas with an L/P ratio lower than normal gastric mucosa were significantly bigger than those having an L/P ratio within normal limits or

higher than normal tissue. In this group the content of protein-bound neuraminic acid was increased, as in the total material, but the content of lipid-bound neuraminic acid, i.e. ganglioside content, was decreased.

Further studies are needed to elucidate the relevance of these findings.

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